Adult corneal epithelium basal cells possess the capacity to activate epidermal, pilosebaceous and sweat gland genetic programs in response to embryonic dermal stimuli

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SUMMARY

Recent work has shown remarkable plasticity between neural and hematopoietic, as well as between hematopoietic and muscle stem cells, depending on environmental stimuli (Fuchs, E. and Segre, J. A. (2000) Cell 100, 143-155). Stem cells give rise to a proliferative transient amplifying population (TA), which is generally considered to be irreversibly committed. Corneal epithelium provides a particularly useful system for studying the ability of TA cells to activate different genetic programs in response to a change in their fibroblast environment. Indeed, corneal stem and TA cells occupy different localities – stem cells at the periphery, and TA cells more central (Lehrer, M. S., Sun, T. T. and Lavker, R. M. (1998) J. Cell Sci. 111, 2867-2875) – and thus can be discretely dissected from each other. It is well known that pluristratified epithelia of cornea and skin display distinct programs of differentiation: corneal keratinocytes express keratin pair K3/K12 and epidermal keratinocytes keratin pair K1-2/K10; moreover, the epidermis forms cutaneous appendages, which express their own set of keratins. In our experiments, central adult rabbit corneal epithelium was thus associated either with a mouse embryonic dorsal, upper-lip or plantar dermis before grafting onto nude mice. Complementary experiments were performed using adult mouse corneal epithelium from the Rosa 26 strain. The origin of the differentiated structures were identified in the first case by Hoechst staining and in the second by the detection of β-galactosidase activity. The results show that adult central corneal cells are able to respond to specific information originating from embryonic dermis. They give rise first to a new basal stratum, which does not express anymore corneal-type keratins, then to pilosebaceous units, or sweat glands, depending of the dermis, and finally to upper layers expressing epidermal-type keratins. Our results provide the first evidence that a distinct TA cell population can be reprogrammed.

Key words: Cornea, Dermo-epidermal interactions, Differentiation, Epidermis, Hair follicle, Sebaceous gland, Sweat gland, Transient cells, Rabbit, Mouse

INTRODUCTION

In mammals, the pluristratified epithelial components of the skin and the cornea which share a common ectodermal embryonic origin have clear differences. Corneal epithelium does not possess the granular cell layer and anucleate stratum corneum typical of epidermis, and they are characterized by the expression of different pairs of keratins: K3/K12 for corneal epithelium and K1-2/K10 for the epidermis (Sun et al., 1983; O’Guin et al., 1987). Furthermore, only the epidermis forms cutaneous appendages such as glands and hair follicles, which express their own set of keratins (Moll et al., 1982; Heid et al., 1986).

In both adult epidermis and corneal epithelium, keratinocyte populations continuously regenerate from stem-cell progenitors that lead, through the combined processes of proliferation and differentiation, to terminally differentiated cells (Potten and Morris, 1988). It is widely accepted that stem cells are a population of slow-cycling cells that are usually found in well-protected areas, and are often located close to a population of rapidly proliferating transient amplifying (TA) cells (among others; Lavker and Sun, 1982; Morris et al., 1985; Barrandon and Green, 1987). Epidermal stem cells are not randomly distributed within the basal layer, but have a patterned distribution that varies with body site: for example, in the scalp they overlie the dermal papillae, whereas in the palm they lie at the tip of the rete ridges (Watt 1998; Jensen et al., 1999). It is important to note that in the epidermis only a few basal cells express the differentiated-type K1/K10 keratins (Reigner et al., 1986). In contrast, the entire basal layer of the central cornea expresses K3/K12 keratins (Schemer et al., 1986; Chaloin-Dufau et al., 1990). Indeed, the corneal stem
and TA cells are not dispersed along the basal layer but occupy distinct localities in this layer. Studies in the rabbit suggested that the stem cells are localized at the periphery of cornea in the limbus (Schermer et al., 1986; Cotsarelis et al., 1989), since limbal basal cells are slow cycling and lack expression of differentiation markers K3/K12. More recent data obtained in mouse show that a hierarchy of TA cells are distributed from the periphery to the centrum of the corneal epithelium, which consequently contains only late TA cells (Lehrer et al., 1998). With regard to hair stem cells, they are located outside the hair matrix: in the bulge, a portion of the upper mouse follicle that is the follicular attachment site of the arrector pili muscle (Cotsarelis et al., 1991; Yang et al., 1993), or in the outer root sheath in a region below the midpoint of the human hair follicle (Rochat et al., 1994). The concept of stem cells does not exclude the eventuality that there exists a hierarchy of progenitor cells that may revert back, may have differential plasticity and may not be restricted to one tissue type.

Until recently, however, tissue-specific stem cells were considered as unipotent, and their derived TA cells as irreversibly committed. New findings have radically altered this concept, at least in relation to stem cells. Thus, neural-derived stem cells produce a variety of blood cells when put into irradiated mice (Bjornson et al., 1999). Likewise, hematopoietic and muscle stem cells, both of mesodermal origin, have remarkable plasticity depending on environmental stimuli (for reviews, see Fuchs and Segre, 2000; Seale and Rudniki, 2000). Moreover, the idea of adult cell reprogramming has gained general acceptance as a result of cloning experiments in which it has been shown that adult nuclei can be reprogrammed to become totipotent when in contact with a zygote cytoplasm (Wilm et al., 1997; Wakayama et al., 1998). The question thus arises as to whether adult epithelial stem cell populations of the interfollicular skin, hair follicle and corneal epithelium are committed to the production of only one cell lineage – epidermal interfollicular stem cells giving rise only to the epidermis, follicular stem cells to hair follicle derivatives and corneal stem cells to corneal epithelium (Miller et al., 1993). Alternatively, are they pluripotent and consequently of equivalent potentialities? In the second hypothesis, the epithelial stem cells may still possess embryonic features and therefore their microenvironment, the fibroblasts with which they are associated, may play a crucial role in their differentiation. Studies of surgical wounds of different depths performed on pig flank show that re-epithelialization, which starts from residual appendageal structures, leads to an ordinary epidermis when originating from hair follicles, and to a thick plantar-like epidermis when originating from the sweat glands (Miller et al., 1998). Thus, whereas all appendage stem cells are able to give rise to an epidermis, some differences may exist between hair and sweat gland stem cells. Likewise, when human keratinocytes from different regions are transplanted onto nude mice, they retain their characteristics and appeared to be controlled by intrinsic programs (Boukamp et al., 1990).

What would happen if the change in the fibroblast environment of the adult epithelial cells was more drastic, that is if they were recombined with embryonic fibroblasts? Indeed, during embryogenesis, the epidermis is, in most cases, dependent upon its associated dermis for its differentiation. Heterotopic dermal-epidermal recombination experiments between mouse plantar, upper-lip and dorsal embryonic skin components (Kollar 1970; Dhouailly 1977a,b) have shown that the choice between a glabrous or a hairy skin, between hair vibrissa and hair pelage-type follicles or between plantar and dorsal epidermis, is dictated by the origin of the dermis. Moreover, keratin analyses in recombinants involving postnatal plantar tissues (Delorme and Dhouailly, 1989) showed that the plantar-type keratin synthesis depends on the origin of the dermis. In the adult, the rat vibrissa follicle has long been employed as an experimental model. Its dermal papilla stays endowed with inductive hair properties of the embryonic dermal condensation, and, when associated with adult epidermis from different sources, even the plantar region (Reynolds and Jahoda, 1992; Jahoda et al., 1993) or the foreskin (Ferraris et al., 1997), is able to induce hair follicle formation.

In the latter experiments, the question remains as to which epithelial cells of the adult epidermis can respond to new dermal influences, since the basal population contains both stem and transient type cells dispersed throughout the epidermis? The adult cornea, however, has unique advantages as an experimental system because stem and transient epithelial cells are regionally segregated. Here, we have investigated the capacity of the epithelial component of adult mammalian central cornea, to follow an alternative differentiation pathway by associating it with an embryonic dermis from a hair or a sweat gland-forming region. As well as having a characteristic keratin expression, the cornea has two other advantages as a source of adult epithelium for cornea/skin recombination-type experiments – it is easy to manipulate, and it has no appendages. Contemporary investigations have underlined the necessity of identifying the origin of responding cells when exploring tissues interactions (Saha et al., 1989). Taking this caveat into account, and in order to identify unequivocally the origin of the differentiated structures, bispecific epithelial-mesenchymal recombinants were performed, involving rabbit adult central corneal epithelium, and embryonic dorsal and plantar mouse dermis. Rabbit nuclei, which display homogeneous chromatin are easy to distinguish from mouse nuclei which have bright spots of condensed chromatin when Hoescht stained (Cunha and Vandenslice, 1984). This system corresponds to the one that has been beautifully developed in birds, between quail and chick (Le Douarin, 1973). Moreover, some experiments were performed using the Rosa 26 mouse strain, which allows cell origin to be distinguished by β-galactosidase staining (Zambrowicz et al., 1997). It should be noted that the rabbit corneal epithelium is thinner (Chaloin-Dufau et al., 1993), pliable and larger than the mouse one, allowing a larger number of recombination experiments to be performed more easily.

We find that embryonic dermis causes the transformation of adult central corneal epithelium, specifically to form an epidermis, and to differentiate either pilosebaceous units, when confronted with a trichogenic embryonic dermis, or sweat glands when confronted with a plantar dermis.

MATERIALS AND METHODS

Animals
The mice and rabbits were from Iffa-Credo and from Elevage.
Isolation of hair- and sweat gland-inducing embryonic dermis

Embryonic trichogenic dermis was obtained from the upper-lip of 12.5-day mouse embryos, and from the back of 14.5-day mouse embryo. Embryonic plantar dermis was dissected from the posterior limbs of 15.5-day mouse embryos. These were chosen because they were the optimal inducing stages in previous findings (Dhouailly, 1977a,b). The dermis was dissociated from its associated epidermis by enzymatic treatment 15 minutes (1.25% trypsin and 2% pancreatin), in Ca²⁺- and Mg²⁺-free Earle’s solution, then stored (10 minutes to 1 hour) in DMEM/20% foetal calf serum until use.

Preparation and grafting of the epithelial/mesenchymal heterospecific recombinants

Only the central part of the adult rabbit cornea was used. This was carefully excised using scissors, leaving a margin of around 5 mm at the edges to avoid any contamination by limbal epithelium. The entire mouse corneal epithelium was excised, leaving just a margin of 1 mm at the edges to avoid any contamination by limbal epithelium. The entire cornea was then stored in DMEM/20% foetal calf serum and incubated according to standard protocols (Harlow and Lane, 1988). For double staining, sections were incubated with the AK10, anti-rat FITC-conjugated antibody (Southern Biotechnology Associates, AL), and finally rhodamine-conjugated mouse monoclonal antibody AK12. After two final washes with PBS, slides were immersed for 1 minute in a 5.10⁻⁷ M Hoechst 33258 solution (Sigma, Ref. B2883), washed twice in PBS and mounted in Mowiol 4.88 anti-fading medium (Calbiochem, Ref. 475904).

RESULTS

Formation of a new basal stratum and of hair buds

The central corneal epithelium of the adult rabbit is a fully differentiated non-keratinizing epithelium consisting of 6 to 7 layers, a particular feature of which is the absence of a granular and of a cornified layer. Another difference with the epidermis is that in the central cornea, all the cells of the basal layer express the differentiating-type pair of keratins, as shown by K12 labelling in corneal epithelium immediately after recombination (Fig. 1A). At the dermal-epidermal junction, Hoechst labelling unequivocally delineated the mouse dermis and rabbit epithelium (Fig. 1A,B). After 3 days, in heterospecific recombinants of adult rabbit corneal epithelium and embryonic mouse dermis from the plantar (15.5-day embryo) or dorsal (14.5-day embryo) regions (n=5), a new basal layer was formed, in which the cells no longer express K12 at a high level (Fig. 1C). After 6 days, specimens comprising 14.5-day dorsal dermis (n=7), all displayed early hair bulbs growing down into the dermis. Hoechst staining clearly defined these early follicle stages as being derived from the corneal epithelium, and the dermal cells condensing underneath (the dermal papilla precursors) as mouse-derived cells (Fig. 1D). At this stage, in all specimens examined, the epithelium expressed K12 suprabasally (Fig. 1E).

Formation of a fully differentiated epidermis and of hair follicles

Recombinants involving adult rabbit corneal epithelium and 12.5-day upper-lip (n=17) or 14.5-day dorsal mouse dermis (n=27) were analyzed after 12 or 21 days. In every case hair follicles formed. In specimens fixed after 21 days of grafting, in 100% of cases a huge amount of hairs were emerging. In sections, the corneal epithelium appeared to be transformed into an epidermis characterized by a granular and a cornified layer, associated with several pilosebaceous units (Fig. 2A). The rabbit origin of the epidermal cells and of the sebocytes and trichocytes, as well as the mouse origin of the dermal cells, were clearly identified by the Hoechst staining (Fig. 2B,C). All the nuclei in the epidermis, hair follicles and sebaceous glands displayed an homogeneous staining (distinctive of rabbit epithelial corneal origin), while the hair follicle dermal papillae and other dermal cell nuclei displayed intranuclear bodies (distinctive of mouse origin). Another way to distinguish between epidermal and dermal cell origin was to use mouse tissues from the Rosa 26 strain. Relatively few successful recombinants were recovered, partly because the mouse...
corneal epithelium, which is stiffer than that of the rabbit, does not adhere well to the dermis, leading in more than 20 cases to only tiny patches of epithelial cells on the dermis. Nevertheless, in three cases (12 days after recombination), a group of pilosebaceous units formed, in which the epithelial cells were clearly expressing β-galactosidase (Fig. 2D,E).

DISCUSSION

In a series of experiments we show here that adult corneal epithelium, under the influence of embryonic skin dermis, undergoes a transition to an epidermal-type phenotype and produces appendages (hair follicles or sweat glands).
Epidermal potentialities of corneal basal cells

In the dermal-epithelial recombination experiments presented, because the nuclei of cells from these two components belong to two different species, they are easily recognizable: Hoechst staining allows easy discrimination of mouse and rabbit cell nuclei. Likewise the X-gal staining of recombinants involving the Rosa mouse strain clearly ruled out contamination of the implanted inductive dermal cells by epidermal cells of the same origin.

While reports of reprogramming of adult (stem) cells are becoming relatively prevalent (Bjornson et al., 1999), there have been few observations of reprogramming of adult epithelia. In one example, adult hair follicle dermal papillae were shown to induce hair follicles when put in contact with a variety of epithelia (Oliver, 1970; Jahoda et al., 1993; Reynolds and Jahoda, 1992; Ferraris et al., 1997). Others have used tissue corresponding to the regional identity of the underlying dermis. In the dermal-epithelial recombination experiments presented, because the nuclei of cells from these two components belong to two different species, they are easily recognizable: Hoechst staining allows easy discrimination of mouse and rabbit cell nuclei. Likewise the X-gal staining of recombinants involving the Rosa mouse strain clearly ruled out contamination of the implanted inductive dermal cells by epidermal cells of the same origin.

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recombination work to study epithelial-mesenchymal interactions in the reproductive system (Cunha, 1994). This group performed pioneering work (Cunha et al., 1980, 1983; Neubauer et al., 1983) which demonstrated mesenchyme-induced changes to epithelial gene expression. Recently, they investigated whether epithelial differentiation could be reprogrammed in a fully differentiated adult human bladder epithelium (Aboseif et al., 1999) and found that neonatal rat seminal vesicle mesenchyme induced normal adult human bladder urothelium to form glandular structures resembling prostate.

All of the cornea recombination work done previously has used embryonic corneas, and some class-specific differences have been reported. Thus corneal epithelium from the chick embryo is able to form scales or feathers when it is associated with either a tarsometatarsal or a dorsal dermis (Coulombre and Coulombre, 1971). However, in this species the corneal epithelium loses this capability after 7 days of incubation, and so may have limited plasticity (Zak and Linsenmayer, 1985). In contrast, corneal epithelium from rabbit embryos of all ages and even neonates can be induced to form hair follicles when associated with a dorsal embryonic dermis (Ferraris et al., 1994). This discrepancy is not surprising since a similar situation is seen with skin epidermis from avian and mammalian species. Indeed, in chick embryos the epidermis of the mid-ventral apertium progressively loses its capacity to form feathers in response to dorsal feather-forming dermis (Sengel et al., 1969), whereas in mammals, even adult skin epidermis can form hair follicles in response to appropriate dermal influences (Oliver, 1970; Reynolds and Jahoda, 1992; Ferraris et al., 1997). It should be emphasized that the entire thickness of chick epidermis participates in the formation of feather or scale buds, whereas only the basal epidermal layer is implicated in hair or sweat gland bud morphogenesis.

Our interspecific recombination experiments showed clearly that signals from embryonic mouse dermis can be recognised by, and elicit transformation of adult rabbit corneal epithelium to epidermis, hair follicles and glands. Having said this, the detailed chain of events, as revealed by the marking of stages in the process, was also important as it provided greater insight into questions relating to stem cell lineages and cell reprogramming. Most researchers working on epithelial stem cells, follow a fairly conventional model of stem cell activity, in which there is a progressive and irreversible transition from stem cell to transient amplifying cells to differentiated phenotype. Through a variety of different approaches, all work on mammals has suggested that for the cornea the putative stem cells reside in the limbal region. In rabbits, Schermer et al. (1986) and Chaloin-Dufau et al. (1990) showed that monoclonal antibodies specific to K3 or K12, respectively, two corneal-type keratins, marked all cell layers of the corneal epithelium (in other words basal and suprabasal layers), but only suprabasal layers of the limbus. In humans, Pellegrini et al. (1999) used cell culture and clonal analysis to investigate proliferative potential of epithelial cells from all over the ocular surface and reported, similarly that corneal stem cells were located exclusively in the limbus. In mice, slow cycling, thymidine label-retaining cells were shown to be segregated in the limbal portion of the eye, while a hierarchy of TA cells occurs from next to the limbus to the centroid of the corneal epithelium (Cotsarelis et al., 1989; Lehrer et al., 1998). This relatively undifferentiated nature of basal cells in the limbus, as well as their slow-cycling state are quoted as further evidence that stem cells reside there. In our experiments

Fig. 4. Formation of new suprabasal strata in adult rabbit corneal epithelium associated with a mouse embryo dermis, 12 (A,B) or 21 (C,D) days after grafting under the kidney capsule. (A-C) Recombinations with a 12.5-day upper lip dermis. (A) After 12 days, the pluristratified epithelium (e) comprises numerous strata characterized by the presence of corneal-type keratin K12 (red). Note that the basal layer (bl), as well as the forming hair follicles (h) are not labelled. A few cells localized at the point of attachment between hair follicles and epithelium already synthesize the epidermal-type keratin, K10 (green). (B) A higher magnification of the base of one of the hair shown in A, allows recognition of the bispecificity of the epithelial and dermal components. (C) After 21 days, a similar recombination as A, shows that the cells containing keratin K12 (red) are shedding, while continuous layers of keratin K10 (green) synthesizing cells has formed. (D) Recombination with a 15.5-day mouse plantar dermis. After 21 days there is still a continuous layer of corneal-type epithelium (K12) overlying newly formed epidermal strata (K10). d, dermis; dp, dermal papilla. All specimens are cryosections with Hoechst staining and indirect immunofluorescent staining with AK12 and AK10 antibodies. Scale bars: 50 μm in A,C; 25 μm in B,D.
great care was taken to remove tissue from only the central region when dissecting the rabbit corneas. Thus from all the available evidence, the rabbit corneal epithelial cells used for our recombinations were a transient amplifying population. The observation that K12 was expressed throughout the epithelium at the time of grafting (time zero) was confirmatory evidence that the corneal epithelium we used was centrally derived and contained no stem cells.

Within a few days of being recombined with trichogenic or plantar mouse dermis, a new, non-K12 expressing basal layer was produced by rabbit cells. In other words the epithelium had formed a less-differentiated layer, taking on a phenotype equivalent to the corneal limbus, or the basal layer of skin epidermis, both of which harbour stem cells. Thus it appears that the first stage of the transformation process may be the restoration of a more primitive or stem cell-like phenotype from amongst a TA cell population or even an already differentiating population. Recently, Lehrer et al. (1998) described modulation of the proliferative abilities of TA and stem cells in the cornea by injury or application of TPA. They showed in particular that in response to wounding or chemical stimulation, stem cells were mitotically stimulated, and transient amplifying cells had an increased number of rounds of amplification. However, the change here is much more fundamental. Potten and Loeffler (1990) and Loffler and Potten (1997), re-examined many aspects of stem cell behaviour and put forward an elegant screw model to describe the process of change from stem cell to a mature, non-dividing phenotype. As part of this model, TA cells, that have left the stem-cell niche, are not irreversibly committed to a terminal differentiation (or maturation) pathway, but are able to revert to being stem cells in the event of stem cells being removed or destroyed. Our experimental observations appear to reflect these ideas very closely and may be supportive evidence for the model.

Our results are summarized in Fig. 5. They suggest that the first step in the transformation of the corneal epithelial basal cells to epidermis/follicular epithelium is their regression to a more primitive cell type. The observation that after a few days, the basal epithelial cells were both participating in hair follicle morphogenesis and generating suprabasal K12-expressing cells was also intriguing. This illustrates clearly that proliferation and differentiation are not mutually exclusive. This is well documented by several studies in the field of oncogenesis. For example epithelial-derived benign tumors can maintain the same expression of keratins as the tissue of origin (Tsubura et al., 1991). This is another element of the screw model (Potten and Loeffler, 1990), which makes the point that differentiation, i.e. expression of a new gene product, is not necessarily linked to functional irreversibility. In our experiments it must be assumed that hair follicle stem cells were also established during the process of follicle morphogenesis. In this context it is significant that normal-looking full pilosebaceous units (hair with associated sebaceous glands) were produced, since the appearance of sebaceous glands (a subset of follicle epithelia) during follicle morphogenesis must be indicative of stem cell attributes. In a recent paper, it was suggested that stem cells are localized throughout the epithelium of developing hair follicles in the embryo (Akiyama et al., 2000), suggesting that all hair bud epithelial cells are potential stem cells, but that subsequently the stem-cell niche becomes progressively limited. The developing follicles induced in the adult corneal epithelium can be considered as similar to embryonic follicles, which contain large numbers of stem cells. There is clearly a close link between hair follicle stem cells and epidermis, since outer root sheath cells of the human hair follicle have been shown to be able to regenerate a fully differentiated epidermis in vitro (Limat and Noser, 1986; Lenoir et al., 1988). Likewise, after skin injury, follicular cells migrate upwards to cover the wound, suggesting that they can serve as a source of skin epidermis (Lenoir et al., 1988). In relation to this, we show here that the first signs of epidermal differentiation (K10 expression) always appeared at the top of the developing hair follicles. Therefore, this indicates that follicles may be the primary source for epidermal homeostasis. This is in accordance with a suggestion by the Barrandon group, according to which hair follicles are the main repositories of epidermal stem cells (Kobayashi et al., 1993; Rochat et al., 1994), a view supported by Taylor et al. (2000).

The fact that adult corneal epithelium formed glandular...
Reiner Schmidt and Dr Hans Schaefer for their continuous interest so by first reverting to a stem-like condition. Interestingly the formation of epidermis in the plantar recombinations was slower. This could be due to the fact that at the time (15.5 days) the embryonic plantar dermis is removed for the experiments, it is much less developed that embryonic dorsal skin dermis (14.5 days). Alternatively it could be that the large number of induced hair follicles provides a larger number of potential epidermal stem cells, in comparison with the limited number of induced sweat glands. Indeed, glands, like hair follicles are thought to be a source of epidermal stem cells (Miller et al., 1998).

Concerning the change in keratin synthesis, we show here a switch from K12 (corneal-type) to K10 (epidermal-type in the different skin regions, including plantar skin) keratin synthesis, which was induced by the embryonic dermis and is in accordance with previous findings. However, we were unable to show the formation of an additional K9 keratin (plantar-type keratin) by the rabbit corneal epithelium associated with plantar dermis (data not shown), perhaps due to the fact that this species, which does not have a distinct footpad, may not have a functional K9 gene. In previous mouse/mouse recombination experiments, adult plantar dermis was shown to be able to induce in an embryonic dorsal dermis not only the formation of foot pads, but also the synthesis of K9. In the reverse association, embryonic dorsal dermis combined with adult plantar epidermis not only led to the formation of hairs, but turned off the K9 gene (Delorme and Dhouailly, 1989). Likewise, although adult sole-derived keratinocytes continued to express, or even re-expressed K9 when grafted on to muscle fascia (Compton et al., 1998), they did not express it when co-cultured with non-palmar fibroblasts (Yamaguchi et al., 1999). In reverse, the same authors showed that non-palmar keratinocytes co-cultured with palmar fibroblasts expressed K9 keratin.

In conclusion, our results show that even in the adult, corneal epithelial cells retain the ability to transform into an epidermis and to produce hair follicles with associated sebaceous glands or foot pads associated with sweat glands when recombined with embryonic mouse hair-forming or plantar dermis, respectively. Moreover we show that the formation of the new pluristratified epidermis originates from the induced hair follicles, which confirms their role as the main repository of epidermal stem cells (Rochat et al., 1994; Taylor et al., 2000). Thus, despite conditions that may modulate their differentiation state in vivo, corneal epithelium and epidermal keratinocytes have not diverged irreversibly during embryogenesis and share common properties, reminiscent of their common embryological origin. Our results provide a first clear indication that a distinct transient amplifying cell population can be reprogrammed, and they imply that they do so by first reverting to a stem-like condition.

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